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14. ABSTRACT

The goal was to develop a catechol-o-methyltransferase knockout (COMTKO)-estrogen receptor/Wnt-1 (ERKO/Wnt-1) mouse model to study the role of estrogen catechol metabolites in mammary tumorigenesis. The aims were: 1) Through a complex genetic crossing, introduce the COMTKO genotype into the ERCO/Wnt-1 mice; 2) Initiate studies to determine the effects of the absence of COMT on several biochemical end points and on mammary gland development and tumorigenesis in the resulting COMTKO/ERCO/Wnt-1 female mice. ERKO/Wnt-1 mice were obtained after notification of award; COMTKO mice were on-hand. Unanticipated difficulties were encountered. Additional breeding pairs were obtained and successful breeding began. Because of the delay, a 1 year extension of the project to 20 August, 2005 without additional funds was requested and granted. Appropriate breeding pairs have now been developed and breeding is ongoing to generate the mice needed for the study.

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Introduction

The main risk factors associated with the development of breast cancer implicate estradiol (E2) as a causative agent[1]. The accepted hypothesis is that prolonged exposure to estrogen leads to persistent enhanced proliferation mediated through the binding of E2 to the estrogen receptor. However, E2 also undergoes cytochrome P450 (CYP)-mediated oxidative biotransformation to catechol metabolites, 2-hydroxy (2-OH) and 4-hydroxy (4-OH) E2[2]. 4-OH E2 in particular has been shown to cause/contribute to estrogen carcinogenesis through further oxidative metabolism to quinones that form adducts with adenine and guanine in DNA and cause oxidative DNA damage through participation in redox cycling processes[2]. These catechol estrogen metabolites are present in mouse mammary gland tissue at concentrations of pmoles/mg tissue[3]. CEs are primarily inactivated by *O*-methylation catalyzed by catechol-*O*-methyltransferase (COMT)[4]. Inhibition of COMT enhanced E2-induced renal tumorigenesis in the Syrian hamster model[4] and DNA damage in human MCF-7 cells[5], suggesting that COMT is highly protective against adverse effects caused by the catechol metabolites of E2. COMT is polymorphic in humans and several, but not all, epidemiology studies have observed that the genotype encoding a low activity COMT is associated with an increased risk for developing breast cancer in certain women[6-8]. These observations implicate the catechol metabolites of E2 in breast cancer causation. Experimental investigation of the extent and mechanisms of contribution of the estrogen catechol metabolites would be greatly facilitated by the availability of an appropriate animal model. Mammary tumor incidence in intact estrogen receptor knockout (ERKO)/Wnt-1 female mice is 20%, whereas it is reduced to 8% in ovariectomized females [9]. These results demonstrated that in the absence of ER expression, estrogen still contributes to mammary tumor formation. Our hypothesis regarding the role of estrogen in these mice is that the catechol metabolites undergo oxidative biotransformation to quinones which then form DNA adducts directly, or oxidative DNA damage indirectly through redox cycling, and subsequently mutations, which contribute to mammary tumor formation. Since COMT is protective, the absence of COMT would be expected to increase the incidence and/or shorten the latency of tumorigenesis in the ERKO/Wnt-1 mice. Thus, the purpose of the research proposed for this Concept Grant was to develop a catechol-o-methyltransferase knockout (COMTKO)-estrogen receptor/Wnt-1 (ERKO/Wnt-1) mouse model for use in studies on the role of estrogen catechol metabolites in mammary tumorigenesis. The aims were: 1) Through a complex genetic crossing, introduce the COMTKO genotype into the ERCO/Wnt-1 mice; 2) Initiate studies to determine the effects of the absence of COMT on several biochemical end points reflecting the effects of the absence of COMT (e.g. estrogen catechol metabolite levels, DNA damage) and on mammary gland development and tumorigenesis in COMTKO/ERCO/Wnt-1 female mice.

Body

Preliminary studies, partly supported by this Concept grant, revealed that mammary tissue from COMTKO mice formed catechol metabolites of E2, but as expected, not the methoxy metabolites whch are formed when COMT is expressed. Furthermore, oxidative DNA damage in COMTKO mice was increased compared to the level observed in COMT wild type mice (See Figure 1).

After notification of award, ERKO/Wnt-1 mice, in a C57Bl/6 background, were obtained from Dr. Richard Santen (University of Virginia); the C57Bl/6 COMTKO mice were on-hand. However, unanticipated breeding difficulties were encountered. The COMTKO mice went through a period where they stopped breeding. Additional breeding pairs were obtained from the originator of the strain at Rockefeller University. By the time the mice were cleared through quarantine and breeding could commence, the project was about 6 months behind schedule. Thus, a request was made and granted for an extension of the project for a second year to August 20, 2005without additional funds.

The breeding scheme is complex. The genotype of the female mice needed to test the hypothesis is catechol-O-methyltransferase knockout/estrogen receptor knockout/Wnt (COMTKO/ERKO /Wnt).

However, ERKO females and males are infertile and Wnt females cannot nurse their young. Thus, the desired females must be obtained from the following breeding pairs: Female COMTKO /$ER^{+/-}$; Male – COMTKO/$ER^{+/-}$/Wnt (Wnt is carried in the males as a single copy).

Key Research Accomplishments

1. The COMTKO genotype was introduced into female $ER^{+/-}$ and male $ER^{+/-}/Wnt^{+/-}$ mice to generate breeding pairs needed to produce mice of the desired genotype. Seven breeding pairs, which are **Female:** COMTKO/$ER^{+/-}$; **Male:** COMTKO/$ER^{+/-}/Wn^{+/-}$, are now in place.

2. Breeding is ongoing. It was expected that $1/16^{th}$ of the pups would be females with the desired genotype of COMTKO/ERKO/Wnt. However, to date, of the 57 pups genotyped, while approximately 3 were expected to be COMTKO/ERKO/Wnt, none have been obtained. Breeding is continuing. Control female mice that are ERKO/Wnt are also being bred.

Reportable Outcomes

None at this time, other than the fact that the desired breeding pairs have been developed.

Conclusion

No conclusions can be drawn at this point. Unexpected problems were encountered in breeding the COMTKO mice that caused an initial delay in progress. Once new breeding pairs of COMTKO mice were obtained, breeding began to develop the breeding pairs needed to produce female mice of the genotype needed. In this sense, the Concept Project has been successful, since the primary goal of developing the desired breeding pairs has been accomplished. It will now be a matter of time for sufficient number (minimum, 30) of COMTKO/ERKO/Wnt female mice to be identified and maintained for 15-18 months in order to determine the effect of the absence of COMT on mammary tumor incidence.

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Figure

Oxidative DNA Damage in COMTKO vs WT Mice